Relationship between ATP hydrolysis and molecular dynamics in co-freeze-dried sugar-ATP mixtures

A. Pandya[1], I. Ermolina[1], R Storey[2], G. Smith[1]

(1)School of Pharmacy, De Montfort University, UK (2)AstraZeneca UK Ltd, Macclesfield, UK.

apandya@dmu.ac.uk

INTRODUCTION

Freeze-drying is a manufacturing technique, used for stabilizing drugs that are otherwise unstable in solution. In some freeze-dried formulations, the active ingredient is present in low concentration and excipents, such disaccharides, are used variously as bulking agents during freeze-drying and for lyoprotection during the storage of freeze-dried materials. The majority of formulations are maintained in the amorphous state below the glass transition temperature in order to preserve the stability of the material. Above this temperature cooperative and long range molecular dynamics underpin chemical and physical changes in the material, such as devitrification (the physical transition from a structurally disordered solid to a crystalline solid) causing loss of lyoprotective function.

Rate constants below the glass transition (sub-T_g) are very different to those above the glass transition (supra-T_g) therefore shelf life determination from accelerated data above T_g is not reliable for estimation of shelf life below T_g . An alternative approach is therefore required.

In this study we use adenosine tri-phosphate (ATP) as a model drug (which degrades by hydrolysis to Adenosine di-phosphate (ADP), Adenosine mono-phosphate (AMP) to demonstrate that when co-freeze dried with a range of disaccharides one obtains differential levels of chemical stability that can be linked to the sub-T_g molecular dynamics of the amorphous disaccharide matrix.

AIM

The aim of this work is to establish the relationships between sub-T_g dynamics the differential levels of lyoprotection afforded to a moisture sensitive model drug substance (ATP).

MATERIALS AND METHODS

3mL aliquots of solutions of 1% w/w ATP with 10% w/w sugar (either trehalose, maltose, or lactose) were freeze-dried in 10 mL glass vials by freezing at -40°C for 2 h; primary drying at -30°C for 60 h; and secondary drying at 20°C for 10 h. Moisture contents of the freeze-dried mixtures (~1.5%) were determined by TGA. HPLC and BDS analysis were also performed before storing the batch of the vials at 40°C for 30 days.

An ion pair reverse phase HPLC method was used [1] with an Agilent Eclipse C18 column with the detector set at wavelength 260nm. For identifying initial concentration of ATP at day zero, three freeze-dried vials were reconstituted with water to its original solution weight and 250µl of this solution was added to 750µl of phosphate buffer. HPLC analysis was repeated at day 4, 8, 15 and 30 days for each co-freeze-dried sugar-ATP formulation.

Isothermal broad band dielectric spectra were recorded between 0.1 Hz and 1 MHz by placing ~300mg freeze-dried sample between two gold-coated electrodes of 25 mm diameter and 1 mm separation, at day 0.

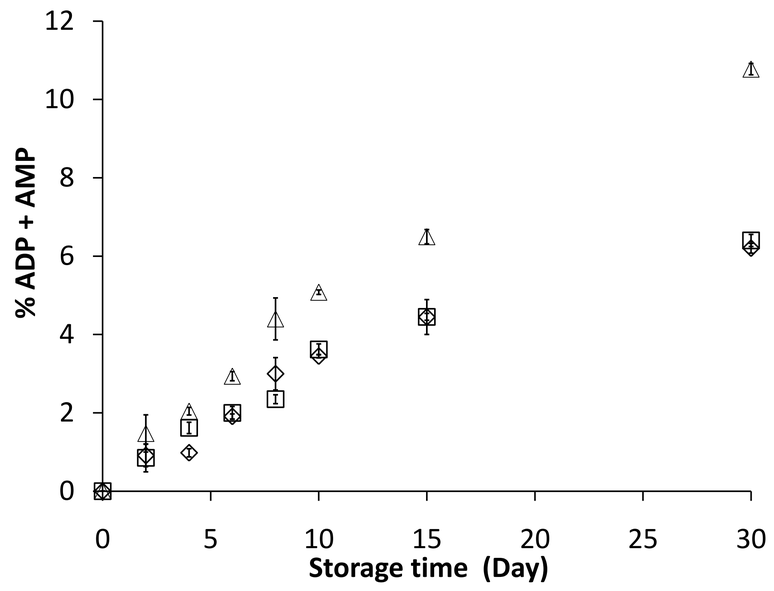

Figure 1 Degradation of ATP into % ADP+ AMP when co-freeze-dried with Lactose (◇), Maltose (□) and Trehalose (△) at 40 °C

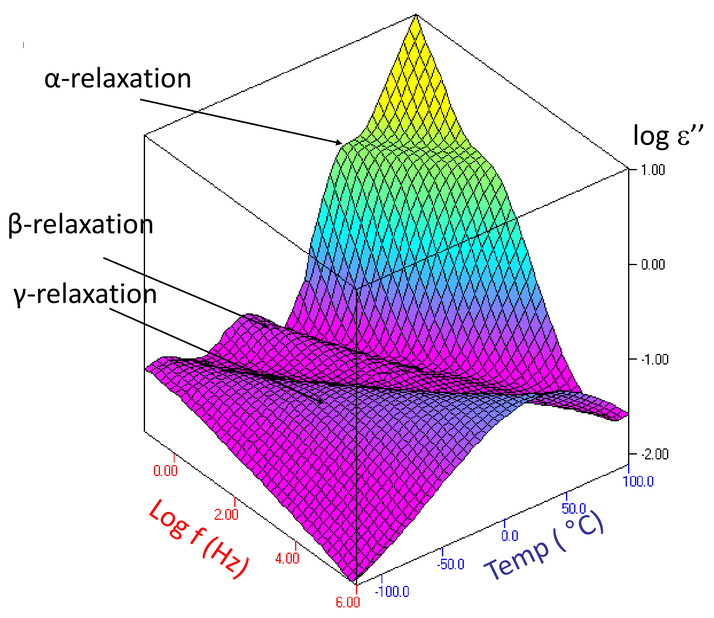

Figure 2 Dielectric permittivity and loss spectra of freeze dried Trehalose + 1% ATP (1.7 % moisture)

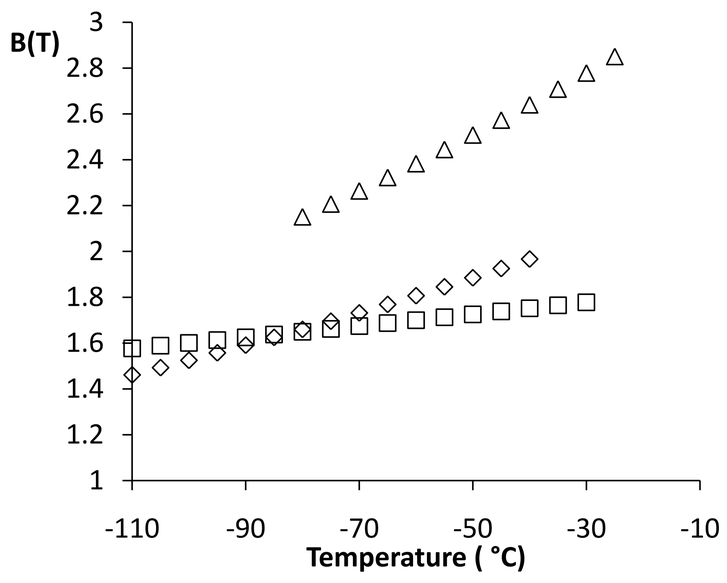

Figure 3 Fröhlich parameter B (T) for dielectric y-relaxation of lactose (◇), maltose (□) and trehalose (△) measured at a range of temperatures.

RESULT AND DISCUSSION

The degradation of ATP was assessed from the area under the curve of the degradation products adenosine di-phosphate (ADP) and adenosine mono-phosphate (AMP). It is noticeable that the degradation of ATP, when co-freeze dried with trehalose, was almost twice that when ATP was co-freeze-dried with either of the other two sugars, maltose and lactose (Figure 1). A similar trend was observed within the molecular dynamics measured in the dielectric relaxation part of this study.

Disaccharides revealed two sub-T_g processes (Figure 2) where each relaxation is associated with small scale mobility (Table 1).

Molecular dynamics were assessed in terms of the dielectric relaxation time (τ) and Fröhlich parameter B(T) for the faster of the two sub-T_g relaxation process (the γ-process). Of these two parameters it was the magnitude of B(T) for relaxation process of freeze-dried sugars at day 0 that followed the trend trehalose>maltose~lactose (Figure 3).

Although the B(T) of sugar-ATP matrix were measured at sub-zero temperatures, and the degradation of ATP was assessed at elevated temperatures, there is a correlation between the trends observed for the three sugars investigated.

The high magnitude B(T) observed for trehalose suggests greater degrees of rotational freedom of hydroxymethyl pendant group [2] which in turn might reflect more generalized degrees of freedom for water diffusion in the sugar matrix which results in the greater hydration/degradation of ATP.

Table 1 Sub-T_g relaxation process by dielectric spectroscopy and their associated molecular mobility below T_g.

Dielectric relaxation	Temperature region (ºC)	Associated mobility
γ -relaxation	-90 to -30	Hydroxymethyl group
β -relaxation	-30 to 20	Glycosidic linkages-boat-chain confirmation

CONCLUSION

The apparent correlation between the rank order of the sugars from the molecular relaxation study and the rank order of the sugars from the ATP stability study is pre-supposed to arise from the fact that the micro-scale relaxation phenomena are at the same scale for water diffusion through the matrix, which is in turn defines the stability of a moisture labile entities such as ATP.

REFERENCE

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